Pestic. Sci. 1997, **51**, 27–32

Determination of Residual 2,4,5-T in Vegetables by High Performance Liquid Chromatography with Ultraviolet and Fluorometric Detection after Derivatization with 2-(2,3-Naphthalimino)ethyl Trifluoromethanesulfonate (NE-OTf)

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(Received 24 May 1996; revised version received 21 October 1996; accepted 22 April 1997)

Abstract: 2,4,5-T was extracted with acetone at below pH 1·0 and the extract was concentrated. After adding 100 g litre^{-1} sodium chloride solution to the residual solution, 2,4,5-T was extracted with ethyl acetate + hexane (20 + 80 by volume). The extract was evaporated to dryness and the residue was dissolved in acetonitrile. 18-crown-6, potassium fluoride and NE-OTf were added to the acetonitrile solution and then allowed to react at 50°C for 20 min. The product was injected to a HPLC with ultraviolet detection operated at 259 nm and fluorometric detection at 394 nm emission and 259 nm excitation. The determination limits of the 2,4,5-T derivative in the sample were 20 μg litre^{-1} with UV detection and 10 μg litre^{-1} with fluorometric detection.

Pestic Sci., **51**, 27–32, 1997
No. of Figures: 6. No. of Tables: 1. No. of Refs: 13

Key words: 2,4,5-T, liquid chromatography, derivatization pesticide, NE-OTf

1 INTRODUCTION

Phenoxy carboxylic acid pesticides, such as 2,4-D, dicamba, MCPA and MCPB have already been determined by GC,[1–8] GC-MS,[9] HPLC[5,10,12] and LC-MS[11] at determination limits of 0·05 μg litre^{-1} to 5 mg litre^{-1}. However, only a few methods for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) have been published so far. In these methods, the residual 2,4,5-T in water was determined at a determination limit of 10 μg litre^{-1} by HPLC,[10] 1·1 μg litre^{-1} by LC-MS[11] and 2 μg litre^{-1} by HPLC with photodiode array detection.[12] As 2,4,5-T has an ultraviolet (UV) absorption maximum near 290 nm, it can be detected by HPLC with UV detection.[10,12] However, extracts of agricultural substrates have many interfering peaks at this wavelength and the sensitivity is relatively low.

Residual 2,4,5-T in agricultural substrates was determined by ECD-GC at a determination limit of 2·5 μg litre^{-1} after butyl esterification.[8] Sensitive methods available in routine laboratory analysis are needed for the monitoring of residual 2,4,5-T in agricultural products.

This report describes the determination of residual 2,4,5-T in vegetables by HPLC with UV and fluorometric (FL) detection, at a limit of 20 μg litre^{-1}, after derivatization with 2-(2,3-naphthalimino)ethyl trifluoromethanesulfonate (NE-OTf), which has been

 Pestic. Sci. 0031-613X/97/$17.50. Printed in Great Britain

reported as a highly reactive ultraviolet and fluorescent agent for carboxylic acids.[13]

2 MATERIAL AND METHODS

2.1 Apparatus

The HPLC system consisted of a JASCO PU-980 pump, a Model 851-AS autoinjecter, a Model UV-980 ultraviolet detector operating at 259 nm, a Model FP-920 spectrofluorometer operating at 394 nm emission and 259 nm excitation, and a Shimadzu Chromatopac C-R6A integrator. A column, Hibar Mightysil RP-18 (4·6 mm × 150 cm, 5 μm) was a gift from Kanto Kagaku (Osaka, Japan). The mobile phase was methanol + water (75 + 25 by volume), at a flow rate of 1·0 ml min^{-1} at 50°C.

2.2 Reagents and materials

Acetone, ethyl acetate, hexane, acetonitrile, methanol, potassium fluoride, sodium chloride, sodium sulfate, hydrochloric acid (Kanto Kagaku Co., Osaka, Japan), 18-crown-6 and NE-OTf, (Wako, Osaka, Japan) were used. (Solid Ne-OTf has been reported to be stable for six months.)[13] 18-Crown-6 (40 mg) and NE-OTf (5 mg) were each dissolved in acetonitrile (10 ml), to give solutions of 4 mg ml^{-1} and 0·5 mg ml^{-1}, respectively. 2,4,5-T (50 mg; Hayashi Junyaku Co., Osaka, Japan) was dissolved in methanol (50 ml) to give a 1 mg ml^{-1} solution. This stock standard solution was diluted with acetonitrile.

2.3 Sample preparation

The sample (10 g) was homogenized in acetone (50 ml) with hydrochloric acid (1 ml) for 3 min and filtered. The residue was washed with acetone (30 ml). The combined filtrate and washings was concentrated to 3–4 ml in a rotary evaporator at 40°C. The residual solution was transferred quantitatively with sodium chloride solution (100 g $litre^{-1}$; 50 ml) to a separatory funnel. Ethyl acetate + hexane (20 + 80 by volume; 100 ml was added) to the separatory funnel, and shaken vigorously for 5 min. After two layers were separated, the upper layer was collected. The above partitioning procedure was repeated with another 30 ml of ethyl acetate + hexane and the upper layers were collected together. The ethyl acetate + hexane layer was dehydrated with sodium sulfate and concentrated to 2–3 ml in a rotary evaporator at 40°C. The residual solution was evaporated to dryness with a gentle stream of nitrogen and the residue was dissolved in acetonitrile (5 ml). An aliquot (0·5 ml) of this solution was pipetted to a test tube and potassium fluoride (20 mg) and 18-crown-6 solution (0·25 ml) were added and mixed. NE-OTf (0·25 ml) solution was then added, mixed and allowed to react at 50°C for 20 min. Out of the resulting 1 ml of solution, 10 μl was applied to HPLC instrument.

3 RESULTS AND DISCUSSION

3.1 Extraction conditions

Initially, the extraction conditions were studied to obtain good recovery of 2,4,5-T.

3.1.1 Effects of pH on extraction

Fifty millilitres of 100 g $litre^{-1}$ sodium chloride solution was adjusted to a pH range of 0·56–5·8 by adding 10, 100 or 380 ml $litre^{-1}$ of hydrochloric acid. One millilitre of the standard solution (5 μg ml^{-1}) was added to each sodium chloride solution above and mixed; the 2,4,5-T was then extracted with ethyl acetate + hexane (20 + 80 by volume; 50 ml). As shown in Fig. 1, at and below pH 4, 2,4,5-T was effectively extracted from the 100 g $litre^{-1}$ sodium chloride solution.

3.1.2 Effect of extraction solvent

Solutions containing 5 μg of 2,4,5-T in 50 ml of 100 g $litre^{-1}$ sodium chloride solution, pH 0·56, were extracted once with 50 ml of hexane, diethyl ether, ethyl acetate and ethyl acetate + hexane (20 + 80 by volume), respectively. When extracted with hexane, the recovery of 2,4,5-T was less than 10%. However, 2,4,5-T was effectively extracted with the other three solvents at

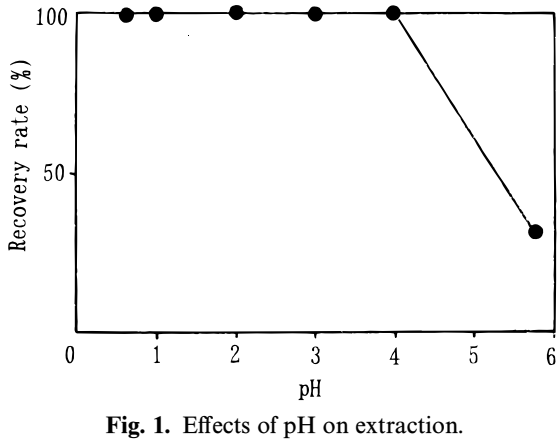

Fig. 1. Effects of pH on extraction.

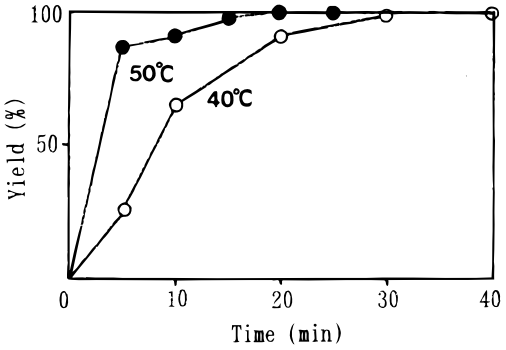

Fig. 2. Effects of reaction time and temperature on derivatization.

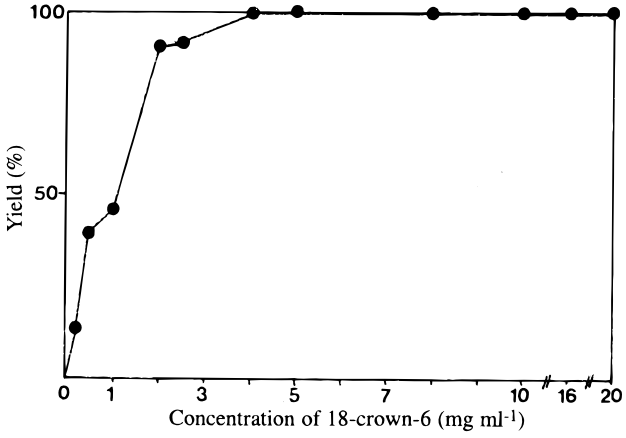

Fig. 3. Effects of concentration of 18-crown-6 on derivatization.

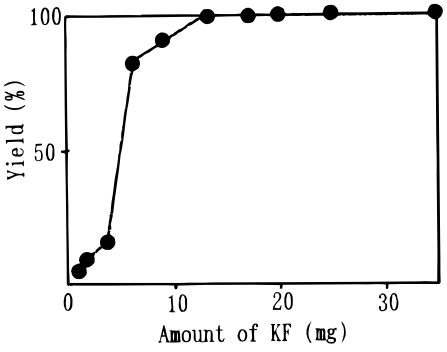

Fig. 4. Effects of potassium fluoride on derivatization.

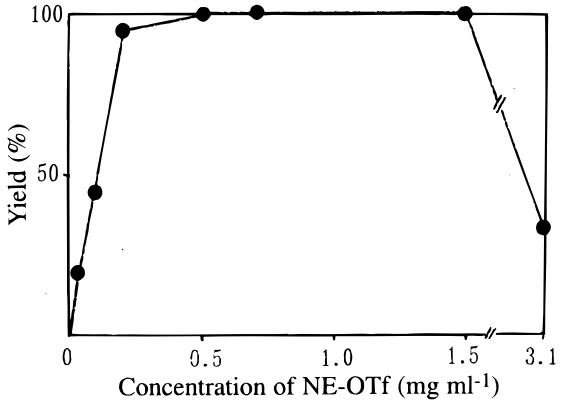

Fig. 5. Effect of concentration of NE-OTf on derivatization.

recovery rates of more than 70%. As extract solvent, ethyl acetate + hexane (20 + 80 by volume) was chosen, because the recovery rate of 78·1% was the best among the tested extract solvents and relatively fewer interfering peaks were observed in the chromatogram of the sample extract.

3.2 Reaction conditions

In the second investigation, reaction conditions were studied to achieve maximum derivatization yield. As a highly reactive ultraviolet and fluorescent labelling agent, NE-OTf was investigated.

3.2.1 Effect of reaction time and temperature on derivatization

18-Crown-6 solution (10 mM; 0·25 ml) and potassium fluoride (5 mg) were added to the standard 2,4,5-T solution (1 μg ml^{-1}; 0·5 ml) and mixed. Then NE-OTf solution (1 mM; 0·25 ml) was added and allowed to react at 40°C or 50°C. As shown in Fig. 2, the reaction time required was >30 min at 40°C and 15 min at 50°C.

3.2.2 Effects of concentration of 18-crown-6 on derivatization

Samples of 18-crown-6 solutions (0·25 ml) containing 0·2 to 20 mg ml^{-1}, were added to the standard 2,4,5-T solution (1 μg ml^{-1}; 0·5 ml) with potassium fluoride (0·5 mg), and mixed. NE-OTf solution (1 mM; 0·25 ml) was added to each of the above mixtures and allowed to react at 50°C for 20 min. As shown in Fig. 3, a concentration of more than 4 mg ml^{-1} of 18-crown-6 was necessary.

3.2.3 Effects of potassium fluoride on derivatization

Potassium fluoride, in a range of 1 to 35 mg, was added to mixtures of 0·5 ml of the standard 2,4,5-T solution (1 μg ml^{-1}; 0·5 ml) followed by 18-crown-6 solution (4 mg ml^{-1}; 0·25 ml) and mixed. NE-OTf solution (1 mM; 0·25 ml) was then added to the above mixtures, and allowed to react at 50°C for 20 min. As shown in Fig. 4, more than 13 mg of potassium fluoride was necessary.

3.2.4 Effect of concentration of NE-OTf on derivatization

18-Crown-6 solution (4 mg ml^{-1}; 0·25 ml) and potassium fluoride (20 mg) were added to 0·5-ml portions of the standard 2,4,5-T solution (1 μg ml^{-1}) and mixed. NE-OTf solution (0·25 ml), with concentrations of 0·05 to 31 mg ml^{-1}, was added to each of the above mixtures and allowed to react at 50°C for 20 min. As shown

in Fig. 5, more than 0·5 mg ml^{-1} NE-OTf was necessary.

3.3 Calibration curves and detection limits

The calibration curves of 2,4,5-T derivatives were linear over the range 0·01 to 1·0 $\mu g\ ml^{-1}$ with a correlation coefficient of 1·0000 by UV detection and from 0·001 to 0·1 $\mu g\ ml^{-1}$ with a correlation coefficient of 0·9999 by FL detection. Although the 2,4,5-T derivative, which is eluted at a retention time of about 10 min, was detected sensitively with FL detection, many peaks were observed around the target peak. As shown in Figs 6A and 6B, the 2,4,5-T derivative was determined by UV detection at a limit of 20 $\mu g\ litre^{-1}$, and by FL detection at a limit of 10 $\mu g\ litre^{-1}$.

3.4 The recovery test

The recovery tests were performed by adding 1 ml of the standard 2,4,5-T solution (0·5 $\mu g\ ml^{-1}$) to 10-g samples. As shown in Table 1, the recovery rates were more than 77%, except in taro, carrot and strawberry. In these three samples, a light emulsion was observed in

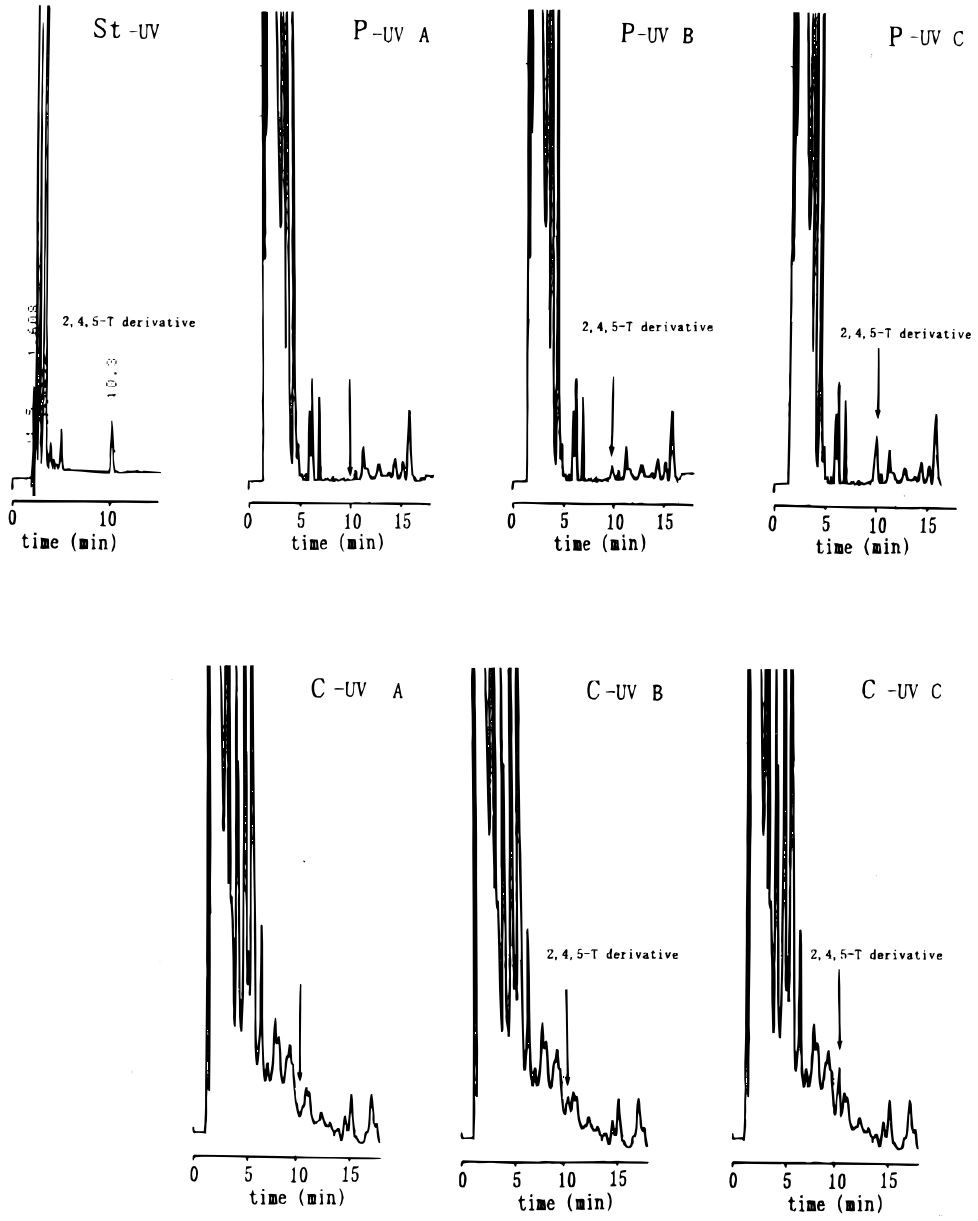

Fig. 6A. Chromatograms of 2,4,5-T derivative and sample extracts detected by ultraviolet detection. St-UV: 0·5 ng of 2,4,5-T derivative detected with ultraviolet (UV) detector. P-UV, C-UV: Chromatograms of Japanese pear (P) and cucumber (C) extracts. A: Sample extracts (Blank), B: Sample extracts with added 0·2 μg of 2,4,5-T. C: Sample extracts with added 0·5 μg of 2,4,5-T (Recovery tests).

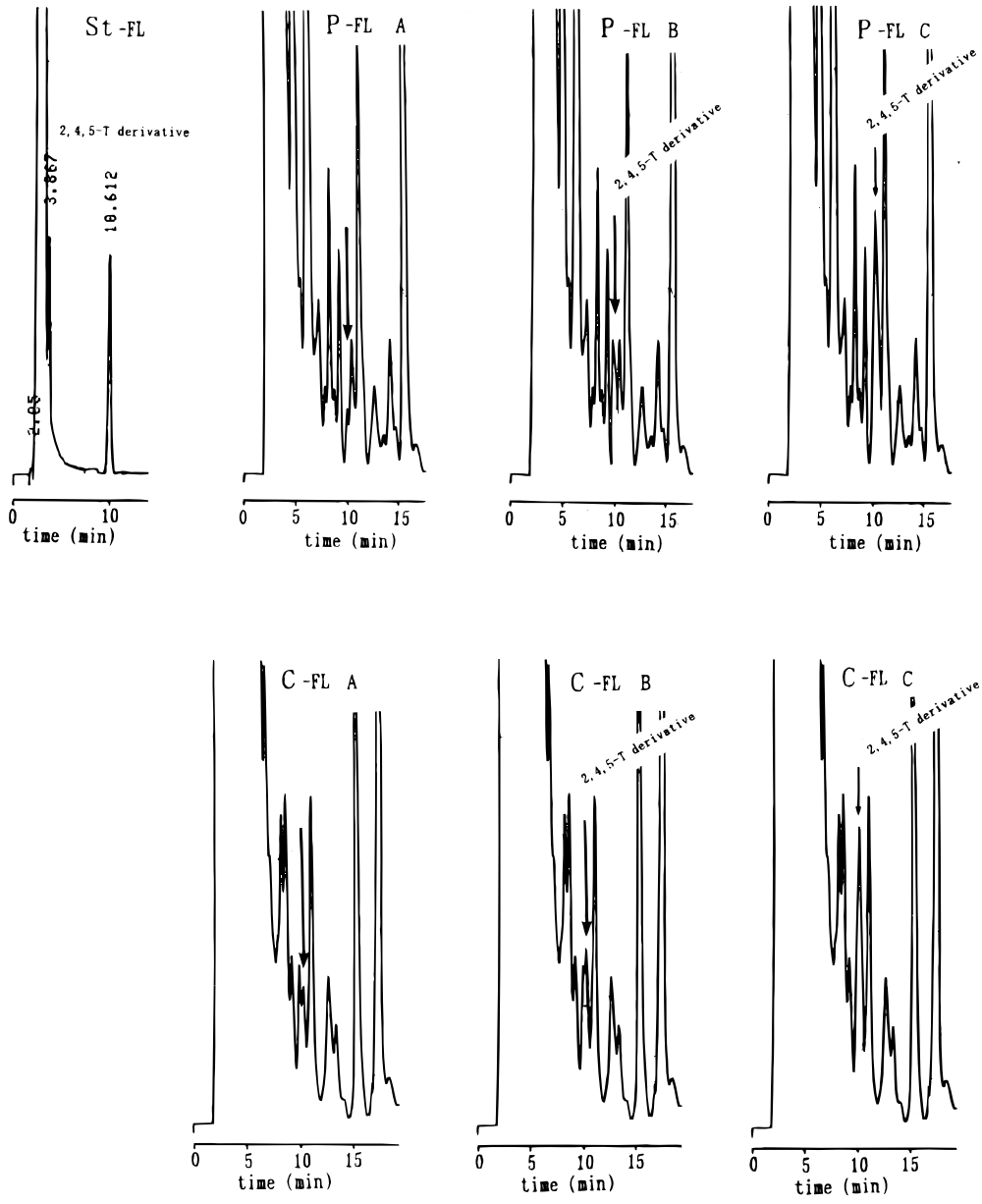

Fig. 6B. Chromatograms of 2,4,5-T derivative and sample extracts detected by fluorometric detection. St-FL: 0·5 ng of 2,4,5-T derivative detected with fluorometric (FL) detector. P-FL, C-FL: Chromatograms of Japanese pear (P) and cucumber (C) extracts. A: Sample extracts (Blank), B: Sample extracts with added 0·1 μg of 2,4,5-T. C: Sample extracts with added 0·5 μg of 2,4,5-T (Recovery tests).

TABLE 1
Results of Recovery Tests

Detection sample	UV		FL	
	Found (μg)	Recovery (%)	Found (μg)	Recovery (%)
Chinese cabbage	0·475	95·0	0·471	94·2
Welsh onion	0·485	97·0	0·459	91·8
Tomato	0·385	77·0	0·389	77·8
Taro	0·307	61·4	0·279	55·8
Cucumber	0·478	95·6	0·491	98·2
Carrot	0·300	60·0	0·327	65·4
Pimento	0·494	98·8	0·474	94·8
Strawberry	0·368	73·6	0·359	71·8
Japanese pear	0·450	90·0	0·475	95·0

One millilitre of 2,4,5-T standard solution (0·5 μg ml^{-1}) was added to 10 g sample. Each recovery is a mean of two trials. Chinese cabbage: cv. Hakusai, Welsh onion: cv. Negi.

the partition of the 100 g $litre^{-1}$ sodium chloride solution with ethyl acetate + hexane (20 + 80 by volume), and it is considered that 2,4,5-T was not extracted effectively by ethyl acetate + hexane in these three cases. Chromatograms of standards and other sample extracts using UV detection and FL detection are shown in Figs 6A and 6B. There were no interfering peaks at the retention time of the 2,4,5-T derivative on the chromatogram of any of the sample extracts by UV detection.

In conclusion, residual 2,4,5-T in vegetables was effectively determined and detected by both UV and FL detection after derivatization with NE-OTf.

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